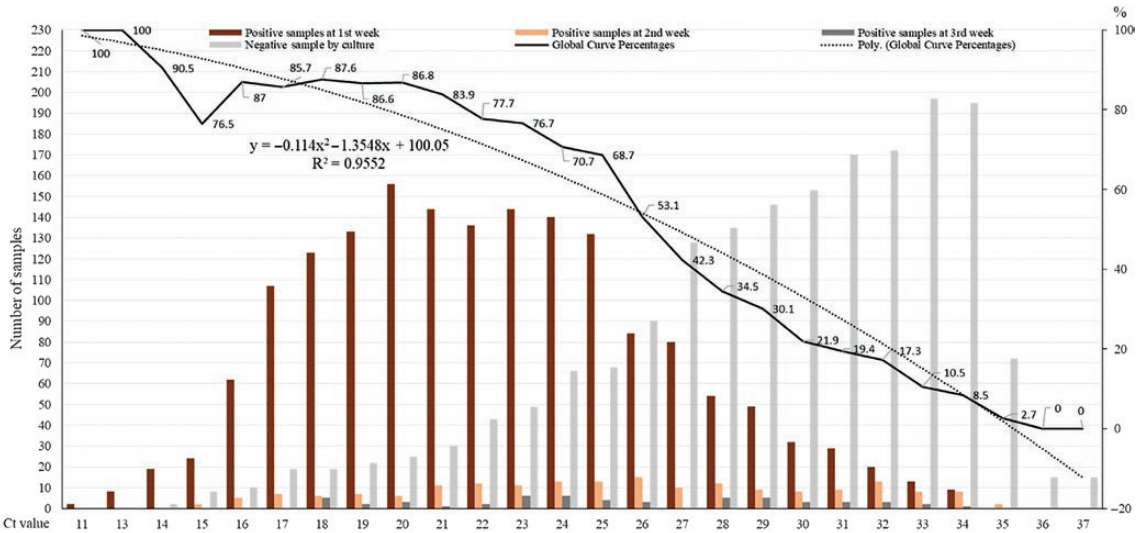
Clinical Infectious Diseases

CORRESPONDENCE

Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates

To the Editor—The outbreak of the coronavirus disease 2019 (COVID-19) pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic on 12 March 2020 by the World Health Organization [1]. A major issue related to the outbreak has been to correlate viral RNA load obtained after reverse-transcription polymerase chain reaction (RT-PCR) and expressed as the cycle threshold (Ct) with contagiousness and therefore duration of eviction from contacts and discharge from specialized infectious disease wards. Several recent publications, based on more than 100 studies, have attempted to propose a cutoff Ct value and duration of eviction, with a consensus at approximately Ct >30 and at least 10 days, respectively [2–5]. However, in an article published in *Clinical Infectious Diseases*, Bullard et al reported that patients could not be contagious with Ct >25 as the virus is not detected in culture above this value [6]. This limit was then evoked in the French media during an interview with a member of the French Scientific Council Covid-19 as a possible value above which patients are no longer contagious [7].

At the beginning of the outbreak, we correlated Ct values obtained using our PCR technique based on amplification of the E gene and the results of the culture [8]. Since the beginning of the pandemic, we have performed 250 566 SARS-CoV-2 RT-PCR for 179 151 patients, of whom 13 161 (7.3%) tested positive. Up to the end of May, 3790 of these samples, reported as positive on nasopharyngeal samples, were inoculated and managed for culture as previously described [8]. Of these 3790 inoculated samples, 1941 SARS-CoV-2 isolates could be obtained after the first inoculation or up to 2 blind subcultures. The correlation between the scanner values and the positivity of the culture allows us to observe that the image obtained with 10 times more isolates than in our preliminary work (1941 vs 129) does not change significantly (Figure 1). It can be observed that at Ct = 25, up to 70% of patients remain positive in culture and that at Ct = 30 this value drops to 20%. At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive. Our Ct value of 35, initially based on the results



Ct value	11	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Number of samples (N = 3790)	2	8	21	34	77	133	153	164	190	186	193	210	225	217	192	218	206	209	196	211	208	220	213	74	15	15
Total positive (n = 1941)	2	8	19	26	67	114	134	142	165	156	150	161	159	149	102	90	71	63	43	41	36	23	18	2	0	0
Week 1: number of positives (n = 1700)	2	8	19	24	62	107	123	133	156	144	136	144	140	132	84	80	54	49	32	29	20	13	9	0	0	0
(% by total positive)	(100)	(100)	(100)	(92.3)	(92.5)	(93.9)	(91.8)	(93.7)	(94.5)	(92.3)	(90.7)	(89.4)	(88.1)	(88.6)	(82.4)	(88.9)	(76.1)	(77.8)	(74.4)	(70.7)	(55.6)	(56.5)	(50.0)	(0)	(-)	(-)
Week 2: number of positives (n = 187)	0	0	0	2	5	7	6	7	6	11	12	11	13	13	15	10	12	9	8	9	13	8	8	2	0	0
(% by total positive)	(0)	(0)	(0)	(7.7)	(7.5)	(6.1)	(4.5)	(4.9)	(3.6)	(7.1)	(8.0)	(6.8)	(8.2)	(8.7)	(14.7)	(11.1)	(16.9)	(14.3)	(18.6)	(22.0)	(36.1)	(34.8)	(44.0)	(100)	(-)	(-)
Week 3: number of positives (n = 54)	0	0	0	0	0	0	5	2	3	1	2	6	6	4	3	0	5	5	3	3	3	2	1	0	0	0
(% by total positive)	(0)	(0)	(0)	(0)	(0)	(0)	(3.7)	(1.4)	(1.8)	(0.6)	(1.3)	(3.7)	(3.8)	(2.7)	(2.9)	(0)	(7.0)	(7.9)	(7.0)	(7.3)	(8.3)	(8.7)	(5.6)	(0)	(-)	(-)
Negative cultured (n = 1849)	0	0	2	8	10	19	19	22	25	30	43	49	66	68	90	128	135	146	153	170	172	197	195	72	15	15

Figure 1. Percentage of positive viral cultures of severe acute respiratory syndrome coronavirus 2 polymerase chain reaction–positive nasopharyngeal samples from coronavirus disease 2019 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve. Abbreviations: Ct, cycle threshold; Poly., polynomial.

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obtained by RT-PCR on control negative samples in our laboratory and initial results of cultures [8], is validated by the results herein presented and is in correlation with what was proposed in Korea [9] and Taiwan [10]. We could observe that subcultures, especially the first one, allow an increasing percentage of viral isolation in samples with Ct values, confirming that these high Ct values are mostly correlated with low viral loads. From our cohort, we now need to try to understand and define the duration and frequency of live virus shedding in patients on a case-by-case basis in the rare cases when the PCR is positive beyond 10 days, often at a Ct >30. In any cases, these rare cases should not impact public health decisions.

Notes

Ethical approval. The protocol was approved by the University Hospital Institute Méditerranée Infection Ethical Committee. All patients provided informed consent in accordance with the Declaration of Helsinki.

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